Rapid Method of Demonstrating Tubercle Bacilli in Sputa.

BY JOHN D. KALES, M.D.
DEMONSTRATOR OF BACTERIOLOGY IN THE MEDICAL DEPARTMENT OF NORTHWESTERN UNIVERSITY.

REPRINTED FROM
THE JOURNAL OF THE AMERICAN MEDICAL ASSOCIATION
APRIL 28, 1894.

CHICAGO:
PRINTED AT THE OFFICE OF THE JOURNAL OF THE ASSOCIATION.
1894.

RAPID METHOD OF DEMONSTRATING TUBERCLE BACILLI IN SPUTA.

Those who have been engaged in the demonstration of bacteria, and the use of the various methods of staining them, have doubtless been prompted to simplify the technique as much as possible. They can thereby attain a much greater percentage of successful preparations when the work is being done by a large class in the laboratory.

A method that is carried out with ease and success by many students working together in a laboratory, must of necessity possess certain advantages that would favor its adoption by every practitioner who realizes the diagnostic value of detecting tubercle bacilli, and has the necessary optical apparatus.

The writer has for several years stained sputa for tubercle bacilli by a rapid method, one quite generally adopted at present. It consisted in using the cover-glass as if it were a dish, and while holding it with a pair of forceps, the various staining fluids were boiled upon its surface, the cover-glass being held in the smokeless flame of an alcohol lamp or Bunson burner. The usual carbol-fuchsin solution was used, and decolorization accomplished by any of the well-known means, *i. e.*, Orth's solution[1] or nitric acid and water 1–4. If Orth's solution is used, decolorization is slower and seems to yield preparations which have little tendency to fade.

Recently the following method has been found still

[1] Strong hydrochloric acid, 1 part; alcohol, 30 parts; water, 70 parts.

more convenient. However, in all methods where the flame is applied directly to a cover-glass for the purpose of heating or boiling a staining fluid, there is great need of using only the best quality of glass. This should be very thin, white and well annealed. The whiteness may be determined by looking at the edges of a package of glasses; these should show no greenish tinge. Their brittleness may be estimated by bending one between the fingers; it should stand considerable flexion before breaking. In buying cover-glasses it is necessary to observe these conditions, for a large number that are sold are not fit to use in rapid staining methods where the flame is directly applied.

It is also very important that the forceps used for holding the cover-glass be of the right kind. The best are the curved fixation forceps made for oculists. Their blades are so set that fluids are not drawn between them by capillary attraction. They grasp the cover-glass firmly and may be locked. The teeth are of no use and can be smoothed off with a file.

One should be provided with a saturated alcoholic solution of fuchsin, which is made by adding dry fuchsin to a quantity of alcohol until no more will be dissolved; the excess will settle to the bottom of the fluid and should be allowed to remain there. A pipette bottle is most convenient for this solution, which keeps indefinitely. A 5 per cent. solution of carbolic acid in distilled water should be at hand and is best kept in a pipette bottle also. For decolorizing, a 25 per cent. solution of nitric acid is placed in a wine glass.

Suppose we use a thin, clean and perfectly dry cover-glass of good quality, three-fourths of an inch square, the film of sputum being fixed upon it in the usual manner; then it will be found that twelve drops of the 5 per cent. carbolic acid solution will cover its surface. This is dropped on while the glass is held in the forceps, then with care the fuchsin solution is added, drop by drop. The first two drops

make a blood red precipitate in the carbolic acid solution, the third tends to clear this slightly, while the fourth should cause the precipitate to disappear entirely. Thus when twelve drops of the 5 per cent. carbolic acid are used, four drops of fuchsin will be found necessary. Other sizes of cover-glasses will require a different quantity of the stain, though the proportion between the two fluids will be practically maintained as 4–12 or 1–3. Whatever size glass is used it must be completely covered with the carbolic acid solution, and it is well to add a few extra drops. This will prevent evaporation leaving any portion of the glass dry. We may ignore the above proportions and always drop the fuchsin until the precipitate that first forms, clears.

Holding the cover-glass in suitable forceps, the staining fluid is boiled upon its surface, but never long enough to dry on the glass. Wash the glass in a stream of running water, still holding it with the forceps, and then wave it back and forth in the decolorizing solution until all color is lost; this may take twenty or thirty seconds. Wash again in running water, dry with the help of blotting paper and heat. If desired, counter stain with cold saturated aqueous solution of methyl blue; if not, invert the dry glass on a drop of glycerin, balsam or water for examination.

The entire process of fixing, staining, decolorizing, etc., may take from two to four minutes, and during this time the glass has been held continuously in the forceps.

There may be conditions when the application of a cold stain for tubercle bacilli—the cover-glass being left in the fluid for twenty-four hours—would be advantageous. The writer has seldom found such conditions necessary. But the above rapid process, which obviates the necessity for keeping on hand staining solutions liable to deteriorate, or obliges one to make up a stock solution whenever there is a suspicion that the one on hand has grown too old, has its advantages.

The overcoming of these difficulties may induce the general practitioner to avail himself more frequently of that method of detecting tuberculosis which has now grown to have a very considerable importance.